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INDUCTION OF SEAWEED CHEMICAL DEFENSES BY AMPHIPOD GRAZING[1]

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Abstract. Grazing by the generalist amphipod *Ampithoe longimana* induced increased concentrations of defensive secondary metabolites in the brown alga *Dictyota menstrualis* and made the seaweed less susceptible to further attack by the amphipod. Although *A. longimana* preferentially consumes *D. menstrualis,* its feeding rates can be reduced significantly by high concentrations of diterpenoid dictyols produced by the alga. In 1991, *D. menstrualis* from sites with high numbers of *A. longimana* had higher levels of grazing scars, higher concentrations of dictyols, and were less palatable to *A. longimana* than plants from sites with few amphipods. Among-site differences in palatability to amphipods did not correlate with plant differences in protein, nitrogen, or carbon content. Within a site, plants that had apparent amphipod grazing scars were significantly less palatable to *A. longimana* than neighboring undamaged plants. Controlled field experiments manipulating *A. longimana* densities supported the hypothesis that feeding by this amphipod induced elevated chemical defenses in the alga. Compared to undamaged control plants, amphipod-damaged plants had 19–34% more of three diterpenoid secondary metabolites and were 50% less palatable to amphipods. Soluble protein and thallus toughness were unaffected by amphipod grazing and thus could not have caused the differences in palatability. High-pressure liquid chromatography evaluation of adventitious branches growing from blade margins at sites of amphipod grazing scars showed that these branches had significantly elevated levels of two diterpenoids relative to normal blade apices or middles. Thus, the amphipod-induced resistance to further attack occurs through an increase in chemical defenses, and these defenses are, to some extent, localized within the plant thallus. Among-site differences in amphipod densities, grazing scars, seaweed defensive chemistry, and plant palatability that we documented in 1991 varied considerably during 1992 and 1993, suggesting that these interrelationships may be complex. In 1992, *A. longimana* densities did not differ between sites, and there were no between-site differences in palatability or concentrations of deterrent secondary metabolites. In 1993, however, *A. longimana* densities did differ between sites, but between-site differences were less dramatic than in 1991. Some secondary metabolites were slightly, but significantly, increased at the site with higher densities of *A. longimana,* but this had no effect on *A. longimana* feeding.

It has been long recognized that marine herbivores are active participants in seaweed–herbivore interactions and can greatly influence the structure of benthic algal communities. Our findings suggest that seaweeds are not passive participants in these interactions, but can actively alter their susceptibility to herbivores in ecological time. Induced responses to herbivory help explain both spatial (i.e., within-thallus, within-site, and among-site) and temporal variation in the chemical defenses of *D. menstrualis.*

Key words: amphipod; chemical defenses; Dictyota; *induced defenses; intraspecific variation; marine; plant–herbivore interactions; seaweed; terpene; within-plant variation.*

INTRODUCTION

Ecologists are becoming increasingly aware that plants are active participants in the dynamics of plant–animal interactions (Rhoades 1979, 1985, Belsky 1986, Karban and Myers 1989, Tallamy and Raupp 1991, Baldwin 1994). Besides producing nectar, flowers, and fruits to attract animal pollinators and dispersers, plants also produce structures that mimic butterfly eggs (Williams and Gilbert 1981) or grazer scars (Niemelä and Tuomi 1987) to deter herbivores or attract enemies of herbivores, respectively. One of the most commonly documented responses of plants to herbivory has been the induction of increased concentrations of chemical defenses (Rhoades 1985, Karban and Myers 1989, Tallamy and Raupp 1991, Baldwin 1994).

The production of chemical defenses is believed to be costly because defenses use resources that could have been allocated to growth or reproduction (Coley 1986, Herms and Mattson 1992). Constitutive defenses require expenditure of resources even when consumers are absent and the benefits of protection are not realized. In contrast, inducible defenses allow costs of de-

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fenses to be deferred until enemies have been detected, at which time the costs can be offset by the benefits of protection. Induced resistance may therefore be an adaptation that minimizes costs by keeping defenses low until they are needed (Harvell 1990, Baldwin 1994).

The pattern of variation in the chemical defenses of some seaweed species suggests herbivore-induced increases of chemical defenses may be responsible for intraspecific variation in chemical defenses. For example, seaweeds from areas of coral reefs where herbivory is intense often produce more potent and higher concentrations of chemical defenses than plants from habitats where herbivory is less intense (Paul and Fenical 1986, Paul and Van Alstyne 1988). However, clipping experiments have failed to induce increased terpenoid chemical defenses in the green seaweeds *Halimeda, Udotea,* and *Caulerpa* (Paul and Van Alstyne 1992) and clipping or urchin grazing failed to induce higher levels of phlorotannins in the kelps *Ecklonia* and *Alaria* or in the rockweed *Sargassum* (Pfister 1992, Steinberg 1994, 1995). Thus, the higher levels of constitutive chemical defenses from sites with many herbivores could have been generated by preferential grazing, founder effects, selection, or among-habitat variation in other environmental variables. Despite the common occurrence of secondary metabolites in seaweeds (Hay and Fenical 1988, Faulkner 1993 and references therein), there is only one example of an induced increase in the chemical defenses of a seaweed (Van Alstyne 1988). In contrast, there are many examples of inducible chemical defenses in the terrestrial literature as evidenced by the number of reviews on the topic (Baldwin 1994 and references therein). This discrepancy in the prevalence of inducible defenses in terrestrial vs. marine plants could be due to the different biologies of vascular terrestrial plants vs. nonvascular seaweeds (e.g., the induction stimulus from localized damage may not be efficiently translocated in seaweeds), but the lesser amount of research on seaweeds relative to terrestrial plants may also explain much of the disparity.

In our study, we document within-thallus, within-site, among-site, and among-year variation in chemical defenses, toughness, protein content, and susceptibility to herbivory of the chemically defended brown alga *Dictyota menstrualis* and how this variation corresponds to the density of herbivorous amphipods. We also manipulate amphipod density in a controlled field experiment to determine if *D. menstrualis* induces higher levels of chemical defenses in response to amphipod grazing.

Methods

Study sites and organisms

The collection and experimental sites used in this study are located within 8 km of each other near Morehead City and Beaufort, North Carolina, USA. *Dictyota menstrualis* is found intertidally and subtidally at the mudflat at Lennoxville Point and at the seagrass bed at Mitchell Village. The alga is mostly subtidal at Radio Island Jetty, although shallow plants sometimes experience aerial exposure during extreme low tides. Herbivores used in our assays included the sea urchin *Arbacia punctulata* collected from the rock jetty at Radio Island, the pinfish *Lagodon rhomboides* collected near the Morehead City, North Carolina waterfront, and the amphipod *Ampithoe longimana* collected from *Dictyota menstrualis* in grass beds in Bogue Sound, North Carolina.

Dictyota menstrualis in North Carolina produces three diterpenoid secondary metabolites: dictyol E, pachydictyol A, and dictyodial (Cronin et al. 1995). Previous experiments show that pachydictyol A or dictyol E significantly deter feeding by most local fishes, urchins, and mesograzers, but that the amphipod *Ampithoe longimana* is unusually tolerant of these chemical defenses and selectively consumes local species of *Dictyota* (Hay et al. 1987, Duffy and Hay 1991, 1994). The feeding deterrent effects of dictyodial are relatively unstudied due to its chemical instability (Cronin et al. 1995), but indirect assays suggest that it may deter the urchin, but not the fish or amphipod that we studied in this investigation (Cronin and Hay 1996*a*). Thus, natural concentrations of dictyols in *D. menstrualis* are effective defenses against urchins and fishes but are much less effective against the amphipod *A. longimana.* In fact, because fishes rarely visit or consume *Dictyota,* the alga represents a partial refuge from predation for small mesograzers like the amphipod *A. longimana* and the polychaete *Platynereis dumerilii,* which preferentially live on and consume *Dictyota* spp. (Hay et al. 1987, 1988, Duffy and Hay 1991, 1994, Cronin and Hay 1996*a*) despite their defensive chemistry.

Effects of secondary metabolites on herbivore feeding behavior

Dictyol E from *Dictyota* was purified using methods described by Cronin et al. (1995) and its effects on feeding by *Lagodon, Arbacia,* and *Ampithoe* were tested using an agar- and seaweed-based artificial food (see Hay et al. 1994 for methods) that had been used previously to test the effects of secondary metabolites from *Dictyota ciliolata* on these same herbivores (Cronin and Hay 1996*a*). Because *D. ciliolata* contains pachydictyol A, its effects, but not those of dictyol E, had already been determined using this methodology for these herbivores, allowing us to concentrate on dictyol E alone.

General feeding assay procedures

This section explains general methods used to assess the palatability of *Dictyota* to *Arbacia* and *Ampithoe.* Specific details (e.g., number of replicates, duration, and statistical analysis) for each separate feeding assay are described later. Some specifics of the feeding assays

were altered slightly over the 3 yr of this project as we developed improved methodologies. For all feeding assays, herbivores had access to nonassay food until just prior to the assay (i.e., animals were not starved).

Arbacia's willingness to feed on *Dictyota* from different sites was evaluated by offering plants to individual urchins held in 1.8-L plastic tubs with flow-through seawater. Urchins often shred algae making it difficult to determine the plant from which the pieces originated, therefore no-choice feeding assays were performed to avoid placing more than one plant with each urchin. The wet mass of each alga was determined by spinning the seaweed in a salad spinner to remove excess water, weighing the alga to the nearest milligram, and quickly returning the plant to seawater. Each *Arbacia* was offered 200–250 mg wet mass (WM) of *Dictyota* and urchins were allowed to feed for 3–4 d, or until most of the urchins had consumed roughly half their available alga. For each assay plant, a similar portion of the same plant was set up in a similar manner without urchins to control for autogenic changes in mass (Renaud et al. 1990).

The palatability of *Dictyota* to *Ampithoe* was determined using both choice and no-choice feeding assays. Although we felt offering the amphipods a choice between different types of *Dictyota* (e.g., different sites, plant parts, or level of grazing damage) was the better way to assess feeding preferences, no-choice assays were sometimes performed in conjunction with choice assays or when more than two plant types were compared so that we could maintain independence among treatments (Peterson and Renaud 1989). For no-choice feeding assays, ≈100 mg WM of each alga was offered to a separate amphipod in a dish with ≈50 mL of non-flow-through seawater. For assays where *Ampithoe* had a choice of plant types, replicate groups of amphipods were all offered two pieces of algae (≈100 mg WM of each) in a dish with ≈100 mL of seawater; the two pieces were distinguished by sewing threads of slightly different lengths through them. For each assay plant, a portion of the same plant was placed in a separate dish without amphipods to control for changes in mass not due to amphipods.

The amount of *Dictyota* consumed in each replicate was calculated as: $[(H_0 \times C_f/C_0) - H_f]$ where H_0 and H_f were pre-assay and post-assay wet masses of tissue exposed to herbivores and C_0 and C_f were pre-assay and post-assay wet masses of controls for autogenic changes in mass. Replicates in which the alga or herbivore died were excluded from the experiment. The highest number of excluded replicates was 6 of 25. Sample size, whether it was a choice or no-choice assay, and the statistical analysis used for each assay are given below, or in the pertinent figure.

Field sampling of amphipods and grazing damage

The relative abundance of grazing scars on *Dictyota* collected from different sites was determined in November 1991, July 1992, and October 1993. *Ampithoe* appears to be the most important consumer of *Dictyota* at our field sites (Hay et al. 1987, Duffy and Hay 1991, 1994) and it makes small semicircular or circular feeding scars that are distinguishable from other types of damage. Haphazardly selected plants from each site (N = 12–24) were placed in coded bags of seawater and an unbiased observer (i.e., one that did not know the code) scored each plant as being ungrazed, slightly grazed, moderately grazed, or heavily grazed. These scores were given a value of 0, 1, 2, or 3, respectively, and among-site differences in these scores were analyzed with a Kruskal–Wallis test for each date. Because observers assessed grazing damage after looking over all plants collected during a single date, numerical scores were subjectively "scaled" to the range of damage seen for that collection. Scaling of these subjective values was not attempted among dates. Thus, among-site differences for a particular date are valid, but comparisons of numerical scores among-dates should not be done because our scale was relative, not absolute.

To assess amphipod densities on *Dictyota,* 10–20 plants and their associated faunas from each site were carefully sealed in plastic bags while still underwater and returned to the laboratory. After sieving (163-μm mesh) the water from the bag and three freshwater rinses of each plant to retrieve the amphipods, *Ampithoe longimana* were separated from other amphipods. *Ampithoe* and "other amphipods" were counted, and each alga was spun in a salad spinner and weighed to the nearest milligram. Densities per mass of each alga were square-root transformed prior to statistical analysis to decrease the heterogeneity among variances.

Measurement of tissue traits

Plant traits that are potentially important proximal cues for the feeding decisions of herbivores were quantified for plants used in feeding assays. Because herbivores are often nitrogen limited and rarely carbon limited (Mattson 1980), protein is generally considered a good measure of the nutritional quality of plant material. Soluble protein was extracted with 1 mol/L NaOH from freeze-dried tissue and analyzed using the Bradford (1976) method with bovine serum albumin as a standard. The protein data were supplemented by analyzing total nitrogen and carbon with a C/N analyzer for plants collected in November 1991. Concentrations of the three secondary metabolites produced by *D. menstrualis* (dictyol E, dictyodial, and pachydictyol A) were determined using high-pressure liquid chromatography (HPLC) methodologies as described by Cronin et al. (1995).

Temporal and among-site variation in plant traits and in Dictyota*–herbivore interactions*

To evaluate among-site differences in algal palatability, plant characteristics, and how these patterns changed with time and location, haphazardly chosen

plants of *Dictyota* were collected from two or three different sites on five dates spanning a 3-yr period. Most comparisons were made between *Dictyota* collected from a rock jetty at Radio Island and a mudflat at Lennoxville Point; however, *Dictyota* from a seagrass bed at Mitchell Village was included in the initial assessment of among-site variation. These sites were chosen because plants at these locations appeared to differ in grazing damage and associated faunas. Densities of amphipods at the jetty were low, apparently due to predation by fishes (Duffy and Hay 1991), while amphipod densities on plants at the mudflat (Duffy and Hay 1994) and seagrass bed sites seemed high.

On 28–29 October 1991, plants were collected from Radio Island Jetty, Lennoxville Point, and Mitchell Village and kept in flow-through seawater until used in assays within 1 d. The palatability of *Dictyota* from each site was determined by individually offering pieces of 25 separate plants from each site to *Ampithoe* (120 mg WM tissue per amphipod, $N = 25$) or *Arbacia* (200 mg WM per urchin, $N = 25$) in no-choice assays. Amphipods and sea urchins fed for 3.8 and 4.1 d, respectively. The soluble protein ($N = 18$–24), total nitrogen ($N = 8$), total carbon ($N = 8$), and the concentrations of secondary metabolites ($N = 13$–14) in *Dictyota* from each site also were analyzed. The density of amphipods and the relative amounts of grazing scars were determined for 15 individuals of *Dictyota* from each site collected on 8 November 1991. These data were analyzed with a one-way ANOVA and means were compared with a Tukey hsd multiple comparisons test.

On 13 July 1992, *Dictyota* plants were collected in individual plastic bags from the jetty at Radio Island and the mudflat at Lennoxville Point. After assessing amphipod grazing damage ($N = 24$) and amphipod densities ($N = 26$), portions of each plant were used for palatability assays with *Ampithoe* (choice and no-choice; $N = 24$), protein quantification ($N = 8$), and secondary metabolite analysis ($N = 12$). In the no-choice feeding assay, 100 mg WM of each plant was offered to a single amphipod. In the choice feeding assay, 100 mg WM of each plant was offered to a pair of amphipods. Amphipods fed for 2.7 d. The data for each measured variable and no-choice feeding assay were analyzed with two-sample t tests. Data from the choice feeding assays were analyzed with a paired-sample t test.

On 23 September 1992, *Dictyota* was collected again from the jetty and mudflat and its palatability to amphipods determined as described above. Concentrations of soluble protein ($N = 5$–6), dictyol E ($N = 7$–8), dictyodial ($N = 15$), and pachydictyol A ($N = 15$) were evaluated with two-sample t tests.

Collections were made again on 13 July 1993. Between-site differences in palatability of *Dictyota* to *Ampithoe* were determined by offering separate groups of 5–7 amphipods ($N = 24$) a 120 mg WM plant portion from each site. Palatability to *Arbacia* was compared by offering ≈200 mg WM from each plant ($N = 24$ for each site) to individual urchins for 3 d. Concentrations of soluble protein ($N = 15$), dictyol E ($N = 22$), dictyodial ($N = 22$), and pachydictyol A ($N = 17$) were measured for plants from the two sites.

Final collections of *Dictyota* from the jetty and mudflat were made on 25 October (feeding assay and tissue measurement plants) and 26–27 October 1993 (for grazing damage and amphipod density assessment). Feeding assays and plant characteristics were determined as in the previous assays.

Within-site and within-plant variation of D. menstrualis

Differences in the apparency of amphipod grazing scars not only occurred among sites, but also, at times, among plants within a site. By contrasting the palatability of grazed vs. ungrazed plants from a single site, we hoped to determine if previous amphipod damage, rather than between-site differences in physical factors, could be altering the palatability of *Dictyota* to the amphipod *Ampithoe longimana*. From Lennoxville Point, 30 plants with no apparent grazing scars and 30 plants with obvious amphipod grazing damage were collected and assayed for palatability using *Ampithoe*. Groups of 10 amphipods ($N = 23$) were offered a choice of ≈100 mg WM each of ungrazed and previously grazed *Dictyota* for 2.1 d. The remaining seven plants from each treatment were held in similar containers but without amphipods. These served as controls for autogenic changes in mass.

Dictyota often has adventitious branches growing from areas of the thallus that have been damaged by grazing or possibly other factors (G. Cronin and M. E. Hay, *personal observations*). To see if induction is localized to plant parts near damage (i.e., if adventitious branches have more defenses than other tissues), tissue traits of adventitious branches, apices, and the middle of individual plants were compared by collecting 20 individuals from Lennoxville Point that had numerous adventitious branches. Each plant was dissected into its respective parts: adventitious branches growing from the damaged margins of plants were excised with scissors, apices were cut from the top 1 cm of branches, and middles were defined as tissue located 2–6 cm below the tips of branches. Half of the middles were cut into pieces similar in size to the adventitious branches and apices to control, to some extent, for the effects of cutting and of size; the remaining half of the middles was not cut into smaller pieces. The palatability of the four tissue types (i.e., adventitious branches, apices, cut middles, and uncut middles) to *Ampithoe* was determined in a no-choice feeding assay with appropriate controls. About 110 mg WM of each tissue type was offered to 20 separate pairs of amphipods for 1.5 d. Because there was no difference in the amount of cut and uncut middles eaten by *Ampithoe*

(mean ± 1 SE, 27 ± 3 vs. 25 ± 2 mg WM, respectively; $P = 0.6$ paired-sample t test; paired by plant; $N = 20$), additional measurements were not performed on the cut middles.

Tissue toughness, nutritive value, and concentrations of secondary metabolites were measured for each plant part. The toughness of the different plant parts ($N = 20$) was measured with a penetrometer as described by Duffy and Hay (1991). The concentration of secondary metabolites and soluble protein was determined for the different parts of eight individuals. Data from the feeding assay and tissue measurements were analyzed with a mixed-model ANOVA (the fixed factor was "plant portion" and the random factor was "individual plant") followed by a Tukey hsd multiple comparisons test for the "plant portion" term.

Manipulation of amphipod grazing on D. menstrualis

To test directly the effect of previous *Ampithoe* grazing on *Dictyota,* we performed field manipulations to alter *Ampithoe* grazing damage on different halves of individual *Dictyota* plants. Thirty relatively undamaged plants were removed from the rocks at Radio Island Jetty, divided in half underwater, entwined in a 10 cm length of three-strand polypropylene rope, and anchored along the west side of Radio Island Jetty where *Dictyota* grew naturally. Dividing plants in half required localized damage near the base of the plant. We assumed that this minimal damage would cause little, if any, induction of chemical defenses; however, tissue near this site was not used for subsequent measurements and any response to this damage should have occurred equally in both halves. One haphazardly chosen half was designated to be attacked by *Ampithoe* while the remaining half was left ungrazed as a control. Grazing damage was manipulated by periodically enclosing the plants in cages with or without added amphipods. The cages were made by stretching nylon stockings over a cylindrical frame (≈10 cm diameter × 20 cm tall) made of plastic-coated copper wire. Plant halves designated to be grazed were placed in the cages with 8–13 amphipods for 1–3 d, producing levels of damage that were visually similar to levels observed at Lennoxville Point. Control plants remained in similar cages without amphipods for the same time period to control for cage effects. For 2–5 d between grazing periods, all plants were placed back on the jetty to allow them to recover under natural conditions. Plants within a pair were separated by 20–30 cm. The experiment was run once in 1993 and once in 1994. In the 1993 experiment, plants were caged on days 0–1, 3–5, and 7–8 of the 16-d experiment. In the 1994 experiment, plants were caged on days 0–2 and 6–8 of the 10-d experiment.

Despite efforts to minimize plant stress, many plants were lost from our marked ropes and overall recovery of plants was modest. The first time the experiment was performed, from 25 October–10 November 1993, we recovered 8 of 30 paired halves. After using some tissues from these plants to determine their palatability to amphipods, only four of the pairs had enough healthy tissue to analyze for secondary metabolites, and only two pairs had enough tissue remaining for protein analysis. The experiment was repeated from 8 to 18 September 1994, and 18 of 30 paired halves were recovered. Immediately after the first experiment in 1993, we used a digitizer to measure amphipod grazing scars and determine the percentage of thallus area removed by amphipods in each of our eight treatment and control plant portions.

The palatability to *Ampithoe* of amphipod-damaged vs. undamaged plant halves was determined by offering groups of four amphipods a choice of 90 mg WM of each plant half. The amphipods from the 1993 experiment fed for 2.0 d ($N = 8$) and the amphipods from the 1994 experiment fed for 1.0 d ($N = 18$). The concentrations of secondary metabolites ($N = 14$) and soluble protein ($N = 14$) were determined as described above. The data from the 2 yr were pooled and analyzed with paired-sample t tests (i.e., paired by plant). Directed P values with $\gamma/\alpha = 0.8$, as suggested by Rice and Gaines (1994), were calculated when testing for differences in palatability and secondary metabolites based on the prediction that previously damaged *Dictyota* would be less palatable and have more chemical defenses than undamaged plants.

RESULTS

Effect of secondary metabolites on herbivore feeding behavior

Dictyol E reduced feeding by the three herbivores that we assayed (Fig. 1). Natural concentrations of dictyol E in *Dictyota menstrualis* range from 0.02 to 0.045% WM (Fig. 2, Cronin et al. 1995). This compound thus deterred the pinfish *Lagodon rhomboides* at only 27–60% of natural concentrations, but deterred the sea urchin *Arbacia* and the amphipod *Ampithoe* only at the highest range of its natural concentration (Fig. 1). Another secondary metabolite in *Dictyota,* pachydictyol A, deters the sea urchin at, or below, natural concentrations, but does not deter the pinfish or the amphipod until concentrations reach 3–7 times natural levels (see Cronin and Hay 1996*a*). Thus, feeding by pinfish is very sensitive to natural levels of dictyol E, feeding by urchins is deterred by natural levels of pachydictyol A, and feeding by the amphipod is relatively insensitive to average concentrations of either compound; however, dictyol E can depress feeding at the highest of natural concentrations. Our field observations are consistent with these findings in that we rarely see fish or urchin grazing scars on *Dictyota,* but amphipod scars are common in some habitats.

The instability of dictyodial (a third dictyol class diterpene produced by *Dictyota*) once it is purified prevented us from obtaining enough pure compound to

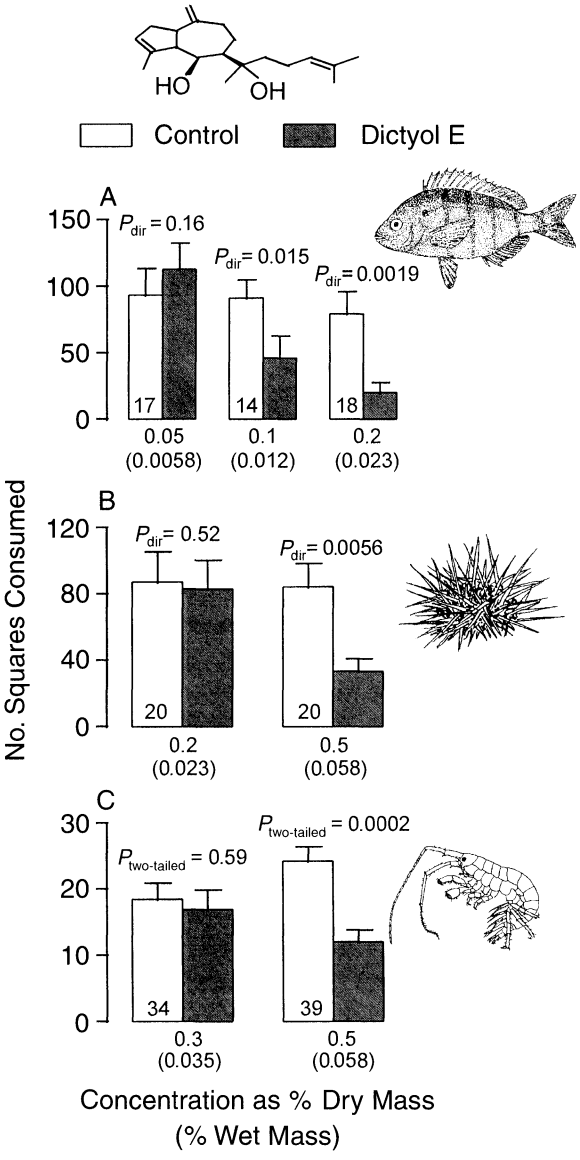

FIG. 1. The effect of dictyol E in choice assays on the feeding behavior of (A) *Lagodon rhomboides,* (B) *Arbacia,* and (C) *Ampithoe.* Sample size is given at the bottom of each control bar. The tested concentrations of dictyol E increase from left to right and are given below each pair of bars as both % DM and % WM. Bars are the mean (±1 SE) amount of food consumed per replicate (i.e., individual pinfish, urchin, or group of 4–6 amphipods). *P* values are from paired-sample *t* tests. Natural concentrations of dictyol E are 0.020–0.045% WM (see Fig. 2).

construct a standard curve in order to determine rigorously its absolute concentrations in our HPLC analysis of *Dictyota.* Relative concentrations (i.e., peak area per tissue mass) of dictyodial, however, varied by a factor of 5–6 among individual plants. Because we currently lack the techniques to prevent dictyodial from degrading during feeding assays, we were unable to test rigorously the effects of this compound on herbivore feeding. Some data suggest that dictyodial might deter the sea urchin but not the pinfish or amphipod (Cronin and Hay 1996*a*).

Temporal and among-site variation in Dictyota*–herbivore interactions*

In 1991, 1992, and 1993, subjective assessments of amphipod grazing scars (i.e., assigned a number of 0–3 that ranged from 0 = not grazed to 3 = heavily grazed) indicated that scars were more common on plants collected from the seagrass bed or mudflat than on plants collected from the jetty ($P < 0.003$ for each year, Table 1). Plants from the mudflat and grass bed habitats were usually ranked as moderately (=2) to heavily (=3) grazed, while jetty plants were usually ranked as undamaged (=0) to little damaged (=1).

Dictyota collected from different sites often differed in nutritive value (e.g., nitrogen and soluble protein), concentrations of secondary metabolites, and/or the number of amphipods associated with the plants (Fig. 2), but differences in these traits often varied among sampling dates. In October–November 1991, *Dictyota* collected from the Mitchell Village seagrass bed, Lennoxville Point mudflat, and Radio Island Jetty had differing estimates of amphipod damage that were positively related to the density of *Ampithoe* and other amphipods on the seaweeds (i.e., Mitchell Village > Lennoxville Point > Radio Island for both measures; Table 1, Fig. 2). In no-choice feeding assays, *Dictyota* plants from the jetty were more susceptible to attack by *Ampithoe* than plants collected from the seagrass bed or mudflat (Fig. 2-1b), but sea urchins did not distinguish among plants from Mitchell Village, Lennoxville Point, and Radio Island (54 ± 13, 78 ± 17, and 66 ± 17 mg consumed per urchin per 4.1 d, respectively; mean ± 1 SE; $P = 0.55$). Because amphipods were significantly deterred by dictyol E at 0.058% WM but not at 0.035% WM (Fig. 1C), the significant 100% higher concentration of dictyol E in mudflat and seagrass bed plants (≈0.045% WM) relative to jetty plants (≈0.022% WM) (Fig. 2-1d) could explain the decreased feeding by *Ampithoe* on the mudflat and seagrass bed plants relative to jetty plants. Pachydictyol A and dictyodial were also significantly higher in the *Ampithoe*-resistant plants from the soft substrate sites (Fig. 2-1d), so avoidance of these plants could have been due to the combined effects of these dictyol class metabolites.

Dictyota from the Mitchell Village seagrass and the Lennoxville Point mudflat bed did not differ significantly in concentration of secondary metabolites (Fig. 2-1d), but plants from Mitchell Village had significantly lower concentrations of soluble protein (Fig. 2-1c), total nitrogen (1.2 ± 0.1 vs. 2.1 ± 0.1% dry mass [DM]), and total carbon (24 ± 1 vs. 28 ± 1% DM). Among-site differences in soluble protein, nitrogen, or carbon content are unlikely to have strongly affected the preference of *Ampithoe* because the more susceptible jetty plants had intermediate levels of sol-

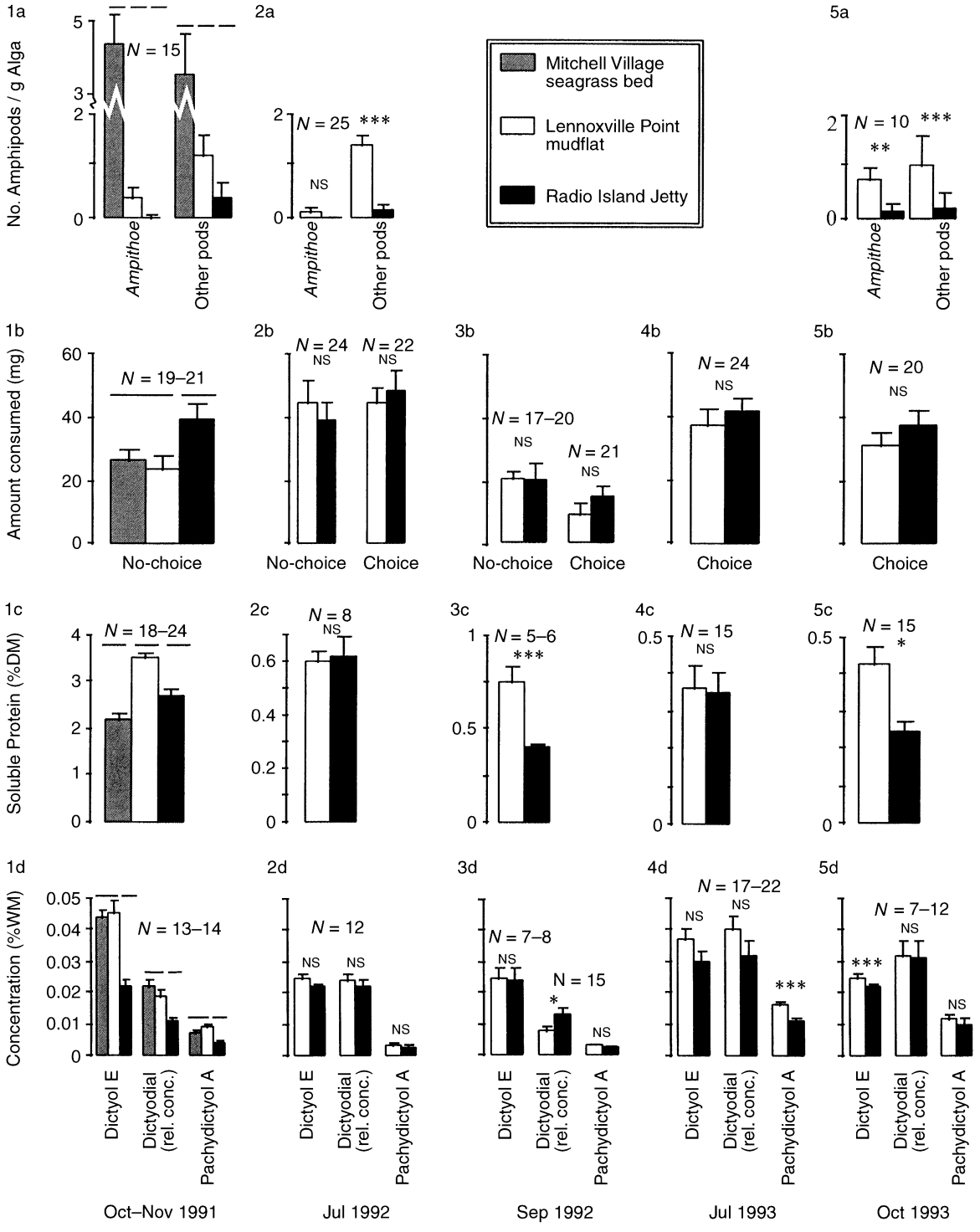

FIG. 2. Among-site and temporal variation of *Dictyota* properties. The (a) density of *Ampithoe* and non-*Ampithoe* ("other pods") amphipods, (b) palatability to *Ampithoe,* (c) soluble protein, and (d) concentrations of diterpenoid secondary metabolites are graphed for *Dictyota* collected from different sites on various dates. Bars represent mean + 1 SE, and sample sizes are given above each set of bars. When three means are compared (i.e., 1a–1d), bars connected by a line do not differ significantly from each other at $\alpha = 0.05$ (Tukey hsd multiple comparison test). Paired-sample *t* tests (for choice feeding assays paired by herbivores) or two-sample *t* tests (all other comparisons) were used to compare parameters for plants collected from Lennoxville Point mudflat vs. Radio Island Jetty (NS = $P > 0.05$; * = $0.01 < P < 0.05$; ** = $0.001 < P < 0.01$; *** = $P < 0.001$).

TABLE 1. Mean subjective numerical rankings of amphipod damage on *Dictyota menstrualis* collected from our different study sites. On a given date, all plants were ranked as undamaged (=0), slightly damaged (=1), moderately damaged (=2), or heavily damaged (=3). Because none of these designations is absolute, observers tend to "calibrate" heavily damaged relative to the most heavily damaged plant in the sample at each time interval. Thus, comparisons among sites within a time interval are valid, but comparisons among time intervals are not. *P* values are from Kruskal–Wallis tests.

Date	Mitchell Village	Lennoxville Point	Radio Island Jetty	*N*	*P*
1991	2.6 ± 0.1 (a)	1.5 ± 0.1 (b)	0.6 ± 0.1 (c)	15	<0.0001
1992		2.8 ± 0.1	1.2 ± 0.2	24	<0.0001
1993		1.7 ± 0.2	0.8 ± 0.2	12–13	<0.003

uble protein (Fig. 2-1c), levels of total nitrogen (1.3 ± 0.1% DM) similar to plants from the seagrass bed, and levels of total carbon (21 ± 1% DM) lower than the other two sites.

The pattern of differences in *Dictyota* between Radio Island Jetty and the Lennoxville Point mudflat observed in October–November 1991 was not observed in July 1992 (Fig. 2-1 vs. 2-2). Densities of *Ampithoe* did not differ between the sites; none were found on the 20 plants collected from Radio Island Jetty, and the number of *Ampithoe* per gram wet mass of alga did not differ significantly from zero for plants from Lennoxville Point. However, grazing scars appeared more abundant on mudflat than jetty plants (Table 1, $P < 0.0001$) and the density of non-*Ampithoe* amphipods was greater at the mudflat than the jetty ("other pods" in Fig. 2-2a). *Ampithoe* exhibited no feeding preference between mudflat or jetty plants whether the seaweeds were offered individually (i.e., no-choice) or simultaneously (i.e., choice) (Fig. 2-2b). Plants from these two sites also did not differ significantly in concentrations of soluble protein (Fig. 2-2c) or secondary metabolites (Fig. 2-2d). Two months later, jetty and mudflat *Dictyota* again did not differ significantly in their susceptibility to *Ampithoe* grazing in either choice or no-choice assays (Fig. 2-3b), although plants from the jetty had 47% less soluble protein (Fig. 2-3c, $P = 0.001$) and 63% more dictyodial than mudflat plants (Fig. 2-3d, $P = 0.025$). As with the July 1992 collection, concentrations of dictyol E and pachydictyol A did not differ significantly between sites (Fig. 2-3d).

In 1993, the susceptibility of mudflat and jetty *Dictyota* to *Ampithoe* did not differ significantly in July (Fig. 2-4b) or October (Fig. 2-5b), although, in October, mudflat plants had more *Ampithoe,* and more non-*Ampithoe* amphipods than jetty plants (Fig. 2-5a) and were subjectively classified as having more amphipod grazing scars (Table 1). However, *Ampithoe* densities at Radio Island Jetty in 1993 were considerably higher than in the previous 2 yr (Fig. 2-a). The susceptibility of *Dictyota* to the urchin *Arbacia* also did not differ significantly in July 1993 (Lennoxville Point vs. Radio Island; 86 ± 19 vs. 102 ± 19 mg consumed per urchin per 3 d; $P = 0.58$). As in the previous year, the amount of soluble protein in *Dictyota* did not differ between sites in July (Fig. 2-4c), but the mudflat plants did have significantly more soluble protein than jetty plants a few months later (Fig. 2-5c). One significant difference in secondary metabolite concentration was detected on both dates in 1993; jetty plants had 31% less pachydictyol A than mudflat plants in July (Fig. 2-4d) and 12% less dictyol E in October (Fig. 2-5d).

Within-site and within-plant variation of D. menstrualis *characteristics*

When undamaged and amphipod-damaged *Dictyota* were collected from the Lennoxville Point mudflat and offered to *Ampithoe* as a choice, the amphipods consumed ≈37% more of the ungrazed than the previously grazed algae (Fig. 3). When these data were analyzed according to Peterson and Renaud (1989), the differences in mass change between damaged and undamaged *Dictyota* in replicates with amphipods ($N = 23$, difference = −11.7 ± 6.6, mean ± 1 SE) vs. replicates without amphipods ($N = 7$, difference = +7.0 ± 2.3) was statistically significant ($P = 0.012$, two-sample t test with separate variances), indicating that *Ampithoe* prefers ungrazed over previously grazed plants (Fig. 3).

The three different portions (apices, middles, and adventitious branches) of amphipod-damaged *Dictyota* from the mudflat demonstrated within-plant variation in toughness, soluble protein, and concentrations of all three secondary metabolites, but this variation did not result in significant differences in susceptibility to grazing by *Ampithoe* when offered in no-choice assays (Fig. 4). Middle portions of plants were significantly tougher than adventitious branches, which were significantly tougher than apices (Fig. 4B). The concentration of soluble protein in adventitious branches did not differ significantly from that of middles or apices; however, apices did have significantly higher concentrations of soluble protein than middles. The within-plant pattern of dictyol E and dictyodial concentrations were similar; adventitious branches had significantly higher concentrations of both compounds than middles or apices, both of which had similar concentrations (Fig. 4D). The concentrations of pachydictyol A in middles and adventitious branches did not differ significantly, but this compound was significantly less concentrated in

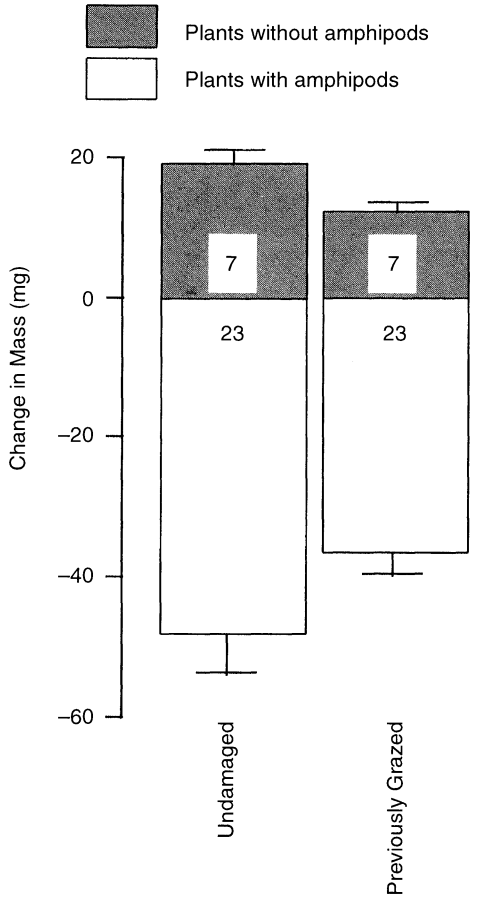

FIG. 3. The amount of undamaged and amphipod-damaged *Dictyota menstrualis* from Lennoxville Point that was consumed by *Ampithoe* in a choice feeding assay. Open bars represent the uncorrected change in mass of the 23 plants consumed by amphipods during the laboratory feeding assay, hatched bars represent the growth of the seven plants not exposed to amphipods during the 2.1-d-long assay (i.e., "correction factor"), and the vertical lines represent 1 SE. The "corrected" amount consumed (i.e., total height of stacked bars) of undamaged vs. amphipod-damaged plants differed significantly ($P = 0.009$) when analyzed with a paired-sample t test, and the changes in mass of the 23 plant pairs grazed during the assay (-11.7 ± 6.6) vs. the seven plant pairs not exposed to amphipods ($+7.0 \pm 2.3$) also differed significantly ($P = 0.012$, two-sample t test).

apices than the other plant parts (Fig. 4D). No significant within-plant difference in the concentration of sterols was detected.

Manipulation of amphipod grazing on D. menstrualis

When grazing by *Ampithoe* was manipulated while other (e.g., physical environment and genotype) factors were controlled, minimally grazed control halves were eaten 62% faster than the heavily grazed plant halves by *Ampithoe* that were offered a choice between the two types of tissue in the laboratory ($P_{dir} = 0.036$, Fig. 5A). Thallus toughness and soluble protein were unaffected by the grazing treatment (Fig. 5B and C). However, heavily grazed plants had significantly higher concentrations of dictyol E (+19%), dictyodial (+22%), pachydictyol A (+34%), and sterols (+9%) (Fig. 5D). During our first effort at this experiment in 1993, plant portions subjected to supplemental amphipod grazing had grazing scars that accounted for $3.3 \pm 1.1\%$ of their total thallus area, while control plant portions lost only $0.2 \pm 0.1\%$ of their thallus area to amphipods ($P_{dir} = 0.013$, paired-sample t test, $N = 8$). We did not quantify grazing scars in 1994 but damage levels appeared similar to those measured in 1993.

DISCUSSION

Mensurative experiments suggest that *Dictyota menstrualis* induces increased levels of chemical defenses in response to grazing by *Ampithoe longimana* (Figs. 2 and 3), the herbivore that appears to be the most important consumer of *Dictyota* at our study sites (Hay et al. 1987, Duffy and Hay 1991, 1994, Cronin and Hay 1996*b*). *Dictyota* collected from sites with 1–2 orders of magnitude higher densities of *Ampithoe* was less susceptible to further attack and had higher concentrations of secondary metabolites than *Dictyota* collected from a site with few *Ampithoe* (Fig. 2-1). When the densities of *Ampithoe* were more similar between sites, the susceptibility of *Dictyota* to *Ampithoe* and levels of chemical defenses were more similar (Fig. 2-2). Between-site differences in *Ampithoe* density and grazing scars, however, did not always result in differences in palatability and chemical defenses (Fig. 2-5). This may occur because grazing thresholds need to be exceeded before induction occurs, or because these between-site and between-year comparisons are complex and affected by factors other than grazing damage by *Ampithoe*. Site-specific variables were eliminated by comparing amphipod-grazed and ungrazed *Dictyota* from within the mudflat site. That some of these plants were ungrazed while neighboring plants had grazing scars was not due to high herbivore resistance in undamaged plants. *Ampithoe* significantly preferred undamaged over amphipod-damaged plants when these were made equally available in laboratory assays (Fig. 3). These observations of lower susceptibility to herbivory of grazer-damaged plants are consistent with the hypothesis that *Dictyota* increases levels of chemical defenses when attacked by amphipods; however, they do not exclude other possible explanations.

A controlled field experiment where individual plants from the jetty were split and subjected to increased densities of *Ampithoe* or to the ambient, low levels of *Ampithoe* found on the jetty demonstrated: (1) that *Dictyota* portions attacked by *Ampithoe* ultimately contained higher concentrations of secondary metabolites than paired portions not attacked by amphipods, (2) that plant portions with these increased chemical defenses were less susceptible to further attack, and (3) that this change in plant susceptibility to

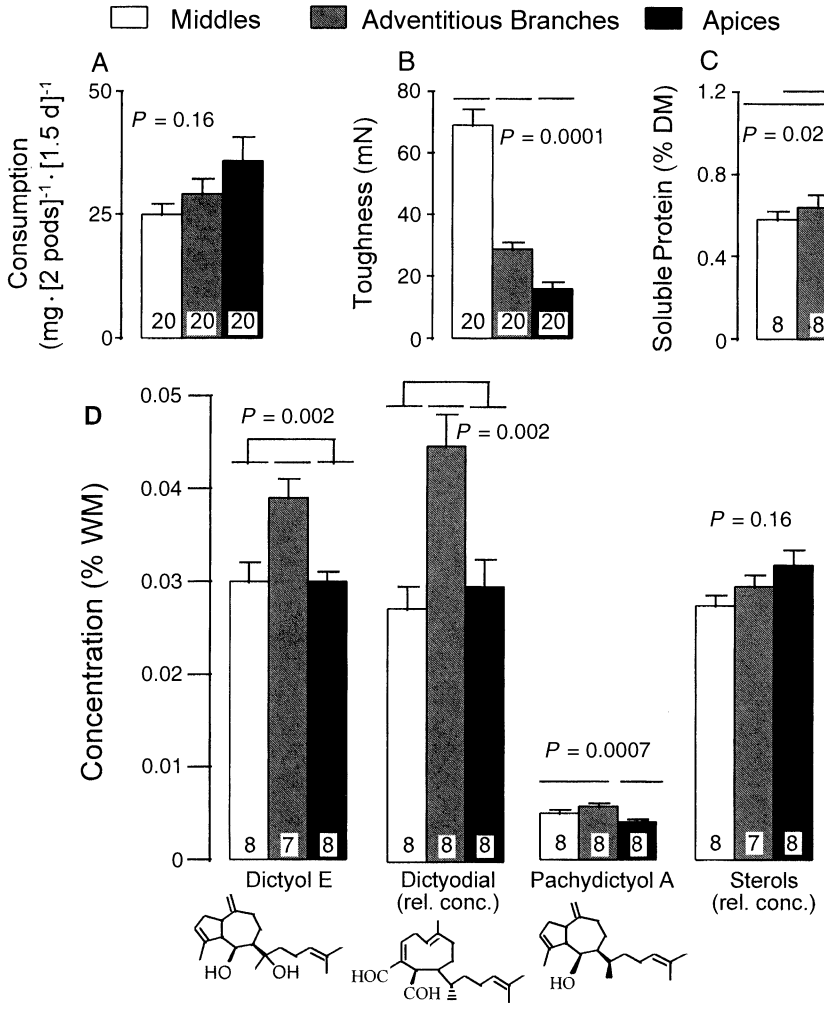

FIG. 4. Within-thallus variation in (A) the susceptibility to *Ampithoe* (no-choice assay), (B) thallus toughness (micro-Newtons), (C) soluble protein, and (D) secondary metabolites and sterols of amphipod-damaged *Dictyota menstrualis* collected from Lennoxville Point. *P* values are from the "plant portion" factor of a mixed-model ANOVA. Means connected by horizontal lines do not differ significantly by Tukey's hsd multiple comparisons test at $\alpha = 0.05$. Other symbols are as in Fig. 1.

amphipods did not occur due to changes in plant toughness or concentrations of soluble protein (Fig. 5). The increase in levels of secondary metabolites in amphipod-damaged plants potentially could have been caused by (1) plants increasing their production of secondary compounds or (2) amphipods selectively removing low-dictyol portions of the plant and leaving portions that were on average higher in secondary metabolites. However, explanation 2 is unlikely because feeding scars on grazed plants recovered in 1993 represented only 3.3% of the surface area in the damaged plants, and only 0.2% of the surface area of control plants. Plants in the 1994 experiment appeared to have similar levels of damage. If we assume that amphipods consumed 3.3% of the experimental plants, and that this 3.3% totally lacked secondary metabolites (extremely unlikely given the pattern in Fig. 4D), then the potential effect of preferential grazing could account for only a 3.4% increase in whole plant concentrations of secondary metabolites. We documented increases of 19–34% in *Dictyota* secondary metabolites (Fig. 5D), indicating that the plants were actually inducing increased levels of defensive metabolites. Induction is also indicated by the finding that adventitious branches growing from damaged areas of the plant produced higher levels of secondary metabolites than did apices (Fig. 4).

Because small structural differences among the secondary metabolites produced by *Dictyota* can have large effects on plant susceptibility to specific herbivores (see review in Hay and Steinberg 1992), it seems possible that the alga could respond to herbivores by preferentially increasing the most effective compound against the currently harmful herbivore. However, when grazed by *Ampithoe, Dictyota* responded by increasing the concentrations of all the secondary me-

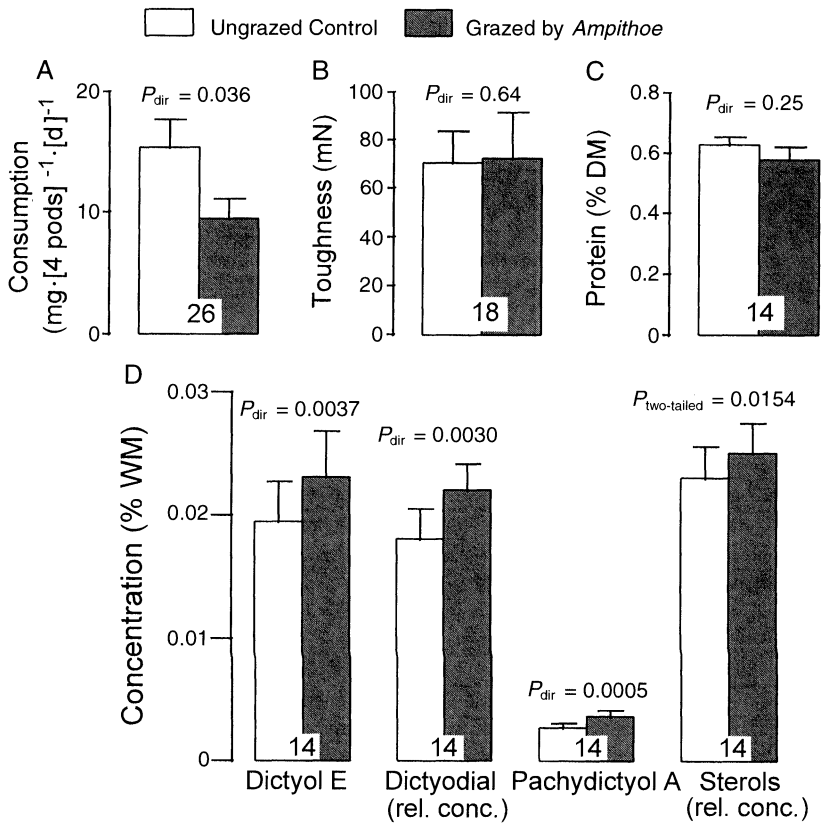

FIG. 5. Differences in (A) the susceptibility to *Ampithoe* (choice assay), (B) thallus toughness, (C) soluble protein, and (D) secondary metabolites and sterols of undamaged control *Dictyota menstrualis* and portions of the same plants that were experimentally exposed to *Ampithoe* grazing in the field. Symbols are as in Fig. 1.

tabolites by 19–34%. In contrast, the primary metabolites we measured (sterols) were increased by only 9%, although this was statistically significant. The alga might have produced this general increase in lipophilic metabolites because it is a general response to thallus damage, because the different secondary compounds may be equally effective against amphipods (e.g., the minimum deterrent concentration assayed with *Ampithoe* was 0.058% WM for both pachydictyol A and dictyol E; Fig. 1C, Cronin and Hay 1996*a*), or as a third possibility, because there could be important synergistic or additive effects of the different compounds affecting feeding by *Ampithoe* (see Hay et al. 1994).

Unlike *Ampithoe,* which has high and similar thresholds for tolerance of both pachydictyol A and dictyol E, other herbivores were more sensitive to small structural differences among *Dictyota* secondary metabolites. For example, dictyol E differs from pachydictyol A only by the substitution of a hydroxyl group on the side branch (compare structures in Fig. 5D), yet dictyol E deterred feeding by the pinfish *Lagodon rhomboides* at 0.023% WM (Fig. 1A) while pachydictyol A did not significantly affect the pinfish at 2.5 times this concentration (Cronin and Hay 1996*a*). The different effects of similar compounds also depend on the herbivore species; 0.023% WM dictyol E did not significantly affect the feeding behavior of the sea urchin *Arbacia* (Fig. 1B), but pachydictyol A significantly deterred urchins at only 56% of this concentration (Cronin and Hay 1996*a*). That *Dictyota* coexists with a diverse herbivore guild (Hay et al. 1987, 1988), of which only a subset is deterred by natural concentrations of any one compound, may explain why the alga produces multiple secondary metabolites.

Differential sensitivity to plant traits among herbivore species may explain why *Dictyota* from three sites were differentially susceptible to *Ampithoe* but not to *Arbacia* in 1991. Although the survivorship of *Ampithoe* is positively correlated with the protein content of food plants (Duffy and Hay 1991), protein content does not appear to be an important proximal cue for their feeding decisions (Duffy and Hay 1991, Cronin and Hay 1996*a, b*). Feeding by the amphipod appears to be more influenced by variation in the concentrations of secondary metabolites than protein (Figs. 2 and 5, Cronin and Hay 1996*a, b*). *Arbacia* is more sensitive than *Ampithoe* to *Dictyota* secondary metabolites (Hay et al. 1987, Cronin and Hay 1996*a*), but the urchin's feeding decisions may also depend on the protein content of food plants (Renaud et al. 1990, Cronin and

Hay 1996*c*). It is possible that *Arbacia* did not feed preferentially on *Dictyota* from the jetty compared to the mudflat because the higher protein content of mudflat plants (Fig. 2-1c) counter balanced the deterrent effects of their chemical defenses. Other studies have demonstrated that the effectiveness of prey defenses can vary according to the nutritional quality of the prey (Duffy and Paul 1992, Hay et al. 1994).

When we used plants collected from the mudflat or plant portions from our induction experiment at Radio Island Jetty, *Ampithoe* avoided previously damaged plants or plant portions compared to undamaged plants or plant portions (Fig. 2-1b, Figs. 3 and 5). These observations, together with our demonstration of significantly greater concentrations of secondary metabolites in previously grazed plants, suggest that amphipods avoid previously damaged plants because these plants are induced to produce greater levels of chemical defenses. This interpretation could be in error if *Ampithoe* avoid plants with amphipod grazing scars as a way of minimizing intraspecific competition or detection by predators that cue on amphipod feeding scars. However, the amphipod did not distinguish between plants that differed in amounts of visible damage (Table 1) but had more similar levels of secondary metabolites (Fig. 2-2 and 2-5). Additionally, artificial foods that were identical except for their concentrations of dictyol E (Fig. 1) or pachydictyol A (Cronin and Hay 1996*a*) were treated differently by *Ampithoe,* with more chemically defended ones being avoided once dictyol E or pachydictyol A concentrations reached 0.058% WM.

Previous studies conflict somewhat with the above findings in that they show that *Ampithoe* preferentially consumes *Dictyota* over other seaweeds and that its feeding is unaffected, or even stimulated, when dictyol E or pachydictyol A are coated onto fresh seaweeds (Hay et al. 1987, Duffy and Hay 1994); however, evenly mixing the compound throughout our agar-based test food demonstrated that feeding by *Ampithoe* can be deterred by relatively high concentrations of these compounds (Fig. 1, Cronin and Hay 1996*a*). Although less affected by *Dictyota* metabolites than urchins and fishes, *Ampithoe* does choose *Dictyota* tissues with lower levels of these secondary metabolites (Fig. 2-5; Cronin and Hay 1996*a, b*). Differences in secondary metabolites between the amphipod-damaged and undamaged halves of our experimental plants is the best supported explanation for their differential susceptibility to *Ampithoe* (Fig. 5). In contrast to these generally consistent patterns of amphipods selecting plants with lower levels of chemical defenses, *Ampithoe* did not avoid chemically rich adventitious branches in contrast to less chemically rich apices and middles (Fig. 4). Thus, secondary chemistry alone is not always sufficient to explain *Ampithoe* feeding choices.

The limited number of investigations evaluating induced chemical defenses in seaweeds suggest that induction may be uncommon. Although both Van Alstyne (1988) and Yates and Peckol (1993) found increased levels of phlorotannins in different species of *Fucus* that they subjected to artificial clipping (Van Alstyne also showed that herbivorous snails fed less on the induced plants), numerous other investigations of both brown and green seaweeds have failed to find evidence of induction (Paul 1992, Paul and Van Alstyne 1992—for terpenoids in *Halimeda, Udotea,* and *Caulerpa*; Pfister 1992—for phlorotannins in *Alaria*; and Steinberg 1994, 1995—for phlorotannins in *Sargassum* and *Ecklonia*). With the exception of Steinberg (1995), all of the above studies used artificial clipping instead of herbivores to damage plants. Because simulated herbivory may not mimic the effects of herbivores (Baldwin 1990, Renaud et al. 1990), studies should be conducted using the herbivores rather than artificial clipping whenever possible. Additionally, most previous studies have searched for induction by measuring the levels of specific secondary metabolites or classes of compounds in damaged vs. undamaged plants rather than by measuring the susceptibility of these plants to relevant herbivores. Because plants could be inducing increased defenses by altering levels of chemical defenses that are not known (and, thus, are not measured) or via plant traits other than chemistry (see Steneck and Adey 1976, Lubchenco and Cubit 1980, Hay 1981, Lewis et al. 1987 for examples), feeding by herbivores may provide a much more sensitive measure of induction than analysis via HPLC or other common chemical techniques. As one possible example, Renaud et al. (1990) found that the brown alga *Padina* became significantly less palatable to sea urchins within 16 h of previous attack; however, they were unable to identify a specific chemical responsible for this effect.

The apparent rarity of induced chemical defenses in seaweeds has been suggested to be due to (1) a greater intensity and spatial predictability of herbivory in marine vs. terrestrial systems, which could select for constitutive rather than inducible defenses (Paul and Van Alstyne 1992, Steinberg 1994), or (2) the difficulty for structurally simple seaweeds (i.e., lacking a vascular system, etc.) of efficiently propagating a signal from the area of grazing damage to the rest of the plant thallus or translocating defenses to the area currently under attack (Cronin and Hay 1996*b*). Alternately, (3) the limited number of seaweed studies that are available may not be representative of the general patterns exhibited by seaweeds, or (4) the common use of artificial clipping instead of herbivores to damage seaweeds may result in an underestimation of induction.

In vascular plants, a systemic induction of defense in response to localized grazing damage could occur via the vascular transport system, although airborne cues may also be important (Baldwin and Schultz 1983). Although most seaweeds lack a vascular system, waterborne cues could elicit a systemic response in seaweeds, as they do in some marine bryozoans (Harvell 1990). This mechanism was probably unimportant

for the induced response observed by Van Alstyne (1988) in *Fucus disticus* because the noninduced, control plants and the induced, damaged plants were separated by only 2–3 cm. The grazed and ungrazed portions of *Dictyota* used in our field experiments were separated by 20–30 cm, so if any waterborne cues were used, they were ineffective at stimulating equal levels of induction in plants that were separated by these distances.

To see if induction of increased defenses in *Dictyota* was localized within the immediate vicinity of the grazing damage or occurred over the entire thallus, we evaluated the concentrations of secondary metabolites in portions of individual thalli that differed in recent history of grazing, as evidenced by adventitious branches growing from what we assumed were amphipod grazing scars. Our assumption that adventitious branches usually resulted from previous amphipod grazing seems reasonable in that (1) adventitious branching from damaged areas of seaweed thalli is common among a variety of different seaweeds (Isaac 1956, Dixon 1958, 1960, Moss 1961, Fulcher and McCully 1969, Van Alstyne 1989), (2) plants with adventitious branches were common at sites where amphipods were abundant and rare where amphipods were rare, and (3) we commonly observed adventitious branches growing from what we recognize as amphipod grazing scars. Adventitious branches of *Dictyota* had elevated levels of dictyol E and dictyodial and intermediate levels of thallus toughness and soluble protein compared to the apices and middles of individual plants (Fig. 4). Although the elevated concentrations of secondary metabolites in adventitious branches are consistent with localized induction of chemical defenses near damaged portions of the thallus, these within-plant differences of *Dictyota* did not result in differential feeding by *Ampithoe* in the laboratory (Fig. 4A). However, levels of secondary metabolites in all portions of the plants used in this experiment were relatively high compared to levels in the jetty plants that were selectively consumed by amphipods (compare Fig. 2-1d and Fig. 4). These patterns suggest that defensive compounds are elevated in new tissues that grow from damaged cells near grazing scars, but that a lesser degree of induction may also occur throughout the entire plant thallus. In our field experiment (Fig. 5) and in the plant portions we analyzed (Fig. 4), the grazing activity of the numerous amphipods may have been diffuse enough that a mosaic of isolated responses produced increased levels of defenses throughout the damaged thalli. Clearly, more research is needed to determine the mechanisms and stimuli responsible for induction in seaweeds.

For a congener of *Dictyota menstrualis* (*D. ciliolata*), *Ampithoe* did respond to within-plant variation in chemical defenses, preferring to consume less chemically rich apices over more chemically rich middles (Cronin and Hay 1996*b*). Australian amphipods feeding on the brown alga *Zonaria angustata* also appear to minimize exposure to its chemical defenses by feeding preferentially on those plant portions that are lowest in the physodes that contain phlorotannins (Poore 1994). Additionally, in a situation similar to ours, Van Alstyne (1989) demonstrated that the brown seaweed *Fucus distichus* produced adventitious branches at herbivore grazing scars and that these adventitious branches were less susceptible to grazing by littorine snails and had higher concentrations of phlorotannins relative to apical meristems. Just as these marine herbivores avoid the chemical defenses of seaweeds they commonly eat, many terrestrial insects avoid the chemical defenses of favored host plants, and perform behaviors like vein cutting or leaf trenching to reduce exposure to the defenses (Dussourd and Denno 1991).

Just as herbivore activity creates intraspecific and interspecific variation in the distribution and abundance of plants, it is becoming increasingly clear that plants actively alter their tissue quality in ways that can affect the distribution of herbivores and enemies of herbivores (Rowell-Rahier and Pasteels 1990, 1992, Schultz 1992). Indirect effects associated with induced changes in *Dictyota* could be important. For example, experiments have shown that *Ampithoe* avoids detection and consumption by omnivorous fishes by being relatively immobile and living on chemically-defended plants that are avoided by fishes (Hay et al. 1987, Duffy and Hay 1991, 1994). If these amphipods, or other herbivores, must increase movement as algal resources change, they could become more susceptible to these visual predators. Alternatively, if grazing induces plant secondary metabolites that the amphipods can tolerate better than the fishes, then plants may become better refuges when grazed. Additionally, the production of adventitious branches increases the morphological complexity of the plants, which could alter the value of plants as refuges from predators (Hacker and Steneck 1990). The active responses of seaweeds to herbivores affect the dynamics of seaweed–herbivore interactions and could influence higher order interactions and have repercussions throughout the community.

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